



**UNIVERSITI PUTRA MALAYSIA**

**INFLUENCE OF CARBOXYPEPTIDASES ON COCOA SPECIFIC AROMA  
PRECURSORS AND METHYLPYRAZINES IN UNDER-FERMENTED  
COCOA BEANS**

**YUSEP IKRAWAN**

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COCOA BEANS**

**By**

**YUSEP IKRAWAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

**June 2003**

*This thesis is specially dedicated to ... ..*

*My beloved wife Sri Widowati and my daughter Zsazsa Nabila*

*for their unconditional patient, love and support*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

**INFLUENCE OF CARBOXYPEPTIDASES ON COCOA SPECIFIC  
AROMA PRECURSORS AND METHYLPYRAZINES  
IN UNDER-FERMENTED COCOA BEANS**

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**June 2003**

**Chairman: Professor Jinap Selamat, Ph.D.**

**Faculty: Food Science and Biotechnology**

A study on the influence of two carboxypeptidases on cocoa specific aroma precursors and methylpyrazines in under-fermented cocoa beans was carried out. Cocoa beans, which were fermented for three days using wooden box were used as samples. The beans were powdered and then incubated at 45°C for 6, 12, 24 and 48 h. Protein content, carboxypeptidase activity, cocoa storage protein, free amino acids, peptide profiles and methylpyrazines concentration were determined. Commercial carboxypeptidase B from porcine and carboxypeptidase Y from baker's yeast were used separately for digestion of cocoa storage protein. Protein content decreased during 3 days of fermentation. Three major bands of cocoa storage protein were found using SDS-PAGE. Carboxypeptidase activity increased after one day, but

later decreased between two and three days of fermentation. During three days of fermentation, free amino acids increased and about 50 to 62% of hydrophobic free amino acids were produced. Hydrophobic peptides in unfermented cocoa beans were considerably higher as compared to under-fermented beans. However, their peptide profiles were very similar for all samples taken at one to three days of fermentation. The concentration of 2,5-dimethyl-, 2,3,5-trimethyl- and 2,3,5,6-tetramethylpyrazines were low after one day and increased during two to three days of fermentation.

The degradation of cocoa storage protein from the samples treated with 5% and 10% of carboxypeptidase B and carboxypeptidase Y revealed similar peptide bands with those of the untreated samples. Hydrophobic free amino acids (alanine, valine, isoleucine, leucine, phenylalanine and tyrosine) were predominantly produced in the samples treated with 5% and 10% of carboxypeptidase B and Y. The peptides profile were very similar with those of the control (well fermented beans). Digestion with carboxypeptidase B and carboxypeptidase Y did not have a significant effect on the concentration of 2,5-dimethyl-, 2,3,5-trimethyl- and 2,3,5,6-tetramethylpyrazines.

Concentrations of acidic amino acid (aspartic, glutamic), hydrophobic amino acids (leucine, alanine, phenylalanine, tyrosine, valine and isoleucine), others (proline, methionine, cysteine, tryptophane, lysine, serine, glycine, histidine,

arginine and threonine) and total of amino acids in the untreated and treated samples with 5% and 10% of carboxypeptidase B and carboxypeptidase Y were significant decreased by roasting at 150°C for 15 min. The proportions of peptides also decreased upon roasting. Peptide profiles revealed very similar chromatogram with those of untreated sample. The concentration of 2,3,5,6-tetramethylpyrazine was significantly higher for samples treated with carboxypeptidase B compared to 2,5-dimethyl- and 2,3,5-trimethylpyrazine. However, the concentration of 2,3,5-trimethylpyrazine was the highest (1727.86  $\mu\text{g } 100\text{g}^{-1}$ ) in the sample with carboxypeptidase B that had been incubated for 24 h. Cocoa liquor made from under-fermented cocoa beans treated with carboxypeptidase B and carboxypeptidase Y were more bitter, astringent, acidic, higher in off-flavour and possessed weaker cocoa flavour compared to the reference (Ghanaian cocoa) sample. There were no significant differences ( $p>0.05$ ) among samples treated with 5% and 10% of carboxypeptidases B and Y; however, there was significant correlation ( $P<0.05$ ) between methylpyrazines and cocoa flavour attribute and also with hydrophobic free amino acids in the samples treated with both enzymes. The overall findings indicate that carboxypeptidase B from porcine pancreas was efficient for the formation of cocoa specific aroma precursors.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KESAN KARBOKSIPEPTIDASE PADA PELOPOR AROMA KOKO  
YANG KHAS DAN METILPIRAZIN PADA KOKO YANG KURANG  
MASA PENAPAIAN**

Oleh

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Suatu kajian keberkesanan dua enzim karboksipeptidase keatas penghasilan pelopor aroma khas koko dan metilpirazin dalam biji koko yang kurang penapaian telah dijalankan. Biji koko tersebut diambil daripada hasil penapaian selama 3 hari. Biji koko dihaluskan dan diinkubasi pada 45°C dengan lama inkubasi yang berbeza yaitu, 6, 12, 24 dan 48 jam. Kandungan protein, aktiviti karboksipeptidase, protein simpanan koko, asid amino, jusuk peptida dan konsentrasi metilpirazin telah diuji. Karboksipeptidase B daripada porcine dan karboksipeptidase Y daripada yis roti digunakan secara terpisah untuk kajian pencernaan. Kandungan protein didapati menurun selama 3 hari penapaian. Tiga jalur utama pada protein simpanan

koko didapati dengan menggunakan SDS-PAGE. Aktiviti karboksipeptidase meningkat daripada 0 sampai 1 hari dan menurun pada 2 sampai 3 hari penapaian. Asid amino meningkat selama 3 hari penapaian dan asid amino hidrofobik dilepaskan diantara 50-62%. Peptida hidrofobik di dalam biji koko yang tidak ditapai (0 hari) didapati sangat tinggi dibanding dengan di dalam biji koko yang kurang penapaian. sempurna. Akan tetapi, jujuk peptida memperlihatkan kesamaan pada 1 sampai 3 hari penapaian. Konsentrasi 2,5-dimetil-, 2,3,5-trimetil- dan 2,3,5,6-tetrametilpirazin didapati rendah setelah 1 hari dan menaik selama 2-3 hari penapaian.

Pemecahan protein simpanan koko daripada sampel-sampel yang diperlakukan dengan 5% dan 10% karboksipeptidase B dan Y menghasilkan jalur protein yang hampir sama dengan sampel tanpa penambahan apapun. Alanine, valine, isoleucine, phenylalanine dan tyrosine adalah asid amino hidrofobik yang dihasilkan secara dominan di dalam sampel-sampel koko yang diperlakukan dengan karboksipeptidase B dan Y. Jujuk-jujuk peptida sangat serupa dengan kontrol (5 hari penapaian). Penambahan karboksipeptidase B dan Y tidak memberikan kesan yang nyata keatas konsentrasi 2,5-dimetil-, 2,3,5-trimetil- dan 2,3,5,6-tetrametilpirazin.

Pemangangan pada 150°C selama 15 min menghasilkan asid amino asidik, asid amino hidrofobik, asid amino lain dan jumlah asid amino di dalam sampel-sampel yang diperlakukan dengan 5% dan 10% karboksipeptidase B



dan Y maupun tanpa perlakuan menurun secara nyata. Jusuk-jusuk peptida hasil perlakuan juga serupa dengan sampel tanpa perlakuan. Konsentrasi 2,3,5,6-tetrametilpirazin di dalam sampel dengan karboksipeptidase B secara nyata lebih tinggi dibanding dengan 2,5-dimetil- dan 2,3,5-trimetilpirazin, akan tetapi konsentrasi 2,3,5-trimetilpirazin sangat tinggi ( $1727.86 \mu\text{g } 100\text{g}^{-1}$ ) di dalam sampel yang telah diinkubasi selama 24 jam dengan penambahan karboksipeptidase B.

Likur koko yang dibuat dari biji koko yang kurang ditapai yang diinkubasi dengan penambahan karboksipeptidase B dan Y mempunyai rasa pahit, kelat, asid, cacat perisa yang lebih tinggi dan perisa koko yang lebih rendah dibandingkan dengan Ghana koko sebagai pembanding. Tidak ada pengaruh nyata ( $p > 0.05$ ) diantara penambahan 5% dan 10% pada kedua enzim. Terdapat hubungan yang nyata antara metilpirazin dan perisa koko dan juga kandungan asid amino hidrofobik di dalam sampel-sampel yang ditreatment dengan kedua enzim. Penemuan ini menunjukkan bahawa karboksipeptidase B dari porcine pancreas lebih berkesan dalam pembentukan pelopor aroma koko yang khas.

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I certify that an Examination Committee met on 5<sup>th</sup> June 2003 to conduct the final examination of Yusep Ikrawan on his Doctor of Philosophy thesis entitled "Influence of Carboxypeptidases on Cocoa Specific Aroma Precursors and Methylpyrazines in Under-fermented Cocoa Beans" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

A handwritten signature in black ink, appearing to read 'Yusep', with a large, stylized loop at the beginning and a horizontal line extending to the right.

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**YUSEP IKRAWAN**

Date: 21 JUL 2003

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